CLAIMS

1. A method of producing a heterologous peptide, polypeptide or protein in a lactic acid bacterium, the method comprising the steps of

(i) constructing a recombinant lactic acid bacterium comprising a nucleotide sequence coding for the heterologous peptide, polypeptide or protein and operably linked thereto, appropriate regulatory nucleotide sequences to control the expression of the coding sequence,

(ii) cultivating said recombinant bacterium under or continuous cultivation conditions to express the gene, and

(iii) harvesting the recombinant bacterium or the peptide, polypeptide or protein.

2. A method according to claim 1 wherein the recombinant cell comprises a constitutive promoter operably linked to the coding sequence.

3. A method according to claim 1 wherein the recombinant cell comprises a regulatable promoter operably linked to the coding sequence.

4. A method according to claim 3 wherein the regulatable promoter is regulated by a factor selected from the group consisting of pH, the growth temperature, the oxygen content, a temperature shift eliciting the expression of a heat chock gene, the composition of the growth medium including the ionic strength and the NaCl content, the presence/absence of an essential cell constituent or precursors therefor, accumulation of a metabolite intracellularly or in the medium, the growth phase of the lactic acid bacterium and the growth rate of the lactic acid bacterium.

5. A method according to claim 3 or 4 wherein the regulatable promoter is derived from a lactic acid bacterium.

6. A method according to claim 5 wherein the regulatable promoter is the pH regulatable P170 promoter disclosed in WO 98/10079 or a derivative thereof which is pH regulatable.

7. A method according to claim 1 or 2 wherein the promoter is introduced into the lactic acid bacterium on an autonomously replicating replicon.

8. A method according to claim 1 or 2 wherein the promoter is a promoter not naturally associated with the nucleotide sequence coding for the heterologous peptide, polypeptide or protein.

9. A method according to claim 1 wherein the heterologous peptide, polypeptide or protein is selected from the group consisting of an enzyme and a pharmaceutically active compound.

10. A method according to claim 1 wherein the coding nucleotide sequence is operably linked to a nucleotide sequence coding for a signal peptide (SP).

11. A method according to claim 10 wherein the signal peptide is selected from the group consisting of the Usp45 signal peptide and the signal peptide having the sequence MKFNKKRVAIATFIALIFVSFFTISSQDAQAAERS (SEQ ID NO: 1).

12. A method according to any of claims 1-4 or 9-11 wherein the lactic acid bacterium is cultivated in a chemically defined medium.

13. A method according to claim 12 wherein the concentration of glucose is kept at a pre-selected concentration of at least about 0.5 g/L by controlled feeding of glucose.

14. A method according to claim 13 wherein the control of feeding of glucose to the medium is linked to pH control.

15. A method according to claim 12 wherein the chemically defined medium is supplemented with yeast extract.

16. A method according to claim 15 wherein the amount of yeast extract is in the range of 0.1-10 g/L.

17. A method according to any of claims 1-14 or 9-11 wherein the yield of heterologous peptide, polypeptide or protein is at least 5 mg/L.

18. A method according to claim 17 wherein the yield of heterologous peptide, polypeptide or protein is at least 100 mg/L.

19. A method according to claim 18 wherein the yield of heterologous peptide, polypeptide or protein is at least 200 mg/L.

20. A chemically defined basal medium (LM1 medium) for cultivating bacteria, the medium comprising:

Component	Concentration, mM or +/-
L-Alanine	3.4
L-Arginine	1.1
L-Asparagine	0.8
L-Cysteine	0.8
L-Glutamate	2.1
L-Glutamine	0.7
Glycine	2.7
L-Histidine	0.3
L-Isoleucine	0.8
L-Leucine	0.8
L-Lysine-HCl	1.4
L-Methionine	0.7
L-Phenylalanine	1.2
L-Proline	2.6
L-Serine	2.9
L-Threonine	1.7
L-Tryptophan	0.5
L-Tyrosine	0.3
L-Valine	0.9
K_2SO_4	0.28[a]
KH_2PO_4/K_2HPO_4	4/6
Na-acetate	15
$CaCl_2$	0.0005[a]

$MgCl_2$	0.52[a]
$FeSO_4$	0.01[a]
Vitamins[b]	+
Micronutrients[a,c]	+
Citric acid	0.1

[a] From Neidhardt et al. J. Bacteriol. **119**:736-747

[b] Vitamins: 0.4 μM biotin, 10 μM pyridoxal-HCl, 2.3 μM folic acid, 2.6 μM riboflavin, 8 μM niacinamide, 3 μM thiamine-HCl and 2 μM pantothenate

[c] Micronutrients: 0.003 μM $(NH_4)_6(MO_7)_{24}$, 0.4 μM H_3BO_4, 0.03 μM $CoCl_2$, 0.01 μM $CuSO_4$, 0.08 μM $MnCl_2$ and 0.01 μM $ZnSO_4$

21. A chemically defined medium (LM3 medium) for cultivating bacteria comprising all the components of the medium of claim 20 in three-fold amounts, except the phosphates and sodium acetate, the respective amounts of which are kept at the same level as in the LM1 medium.

22. A chemically defined medium (LM5 medium) for cultivating bacteria comprising all the components of the medium of claim 20 in five-fold amounts, except the phosphates and sodium acetate, the respective amounts of which are kept at the same level as in the LM1 medium.

23. A chemically defined medium according to any of claims 20-22 comprising glucose in an amount in the range of 1-100 g/L.

24. A method according to claim 12 wherein the chemically defined medium is the medium defined in any of claims 20-23.